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Effect of randomization on the oxidative stability of corn oil

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NOTE TO USERS

Effect of randomization on the oxidative stability of corn oil

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For the Major Program

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ABSTRACT

The oxidative stability of natural and randomized corn oil was studied at high temperature (80 or 100°C) by using an OSI (oxidative stability instrument) and at low temperature (28, 40 or 50°C) by using peroxide values determined by the ferrous iron oxidation test. Randomized corn oil without purification oxidized 1 to 5 times faster than did natural corn oil, depending on the particular lot of corn oil. After alumina-column purification, which removed the tocopherols and other polar compounds, there was no difference in the oxidation rates of the randomized and natural corn oil. These findings suggest that alteration of the glyceride structure had no effect on the oxidation rate of corn oil triacylglycerol (TAG), and that a prooxidant was produced during randomization that can be removed by alumina purification.

The prooxidant had polarity similar to monoacylglycerol (MAG) and diacylglycerol (DAG) on TLC (thin layer chromatography) plates. Synthetic linoleate MAG and DAG showed a slight prooxidant effect in purified corn oil at > 0.25% for MAG and > 5% for DAG, but had no prooxidant effect at similar levels in unpurified oil. The prooxidant effect produced in randomized oil could be greatly increased by trace amounts of cupric or ferrous ions. EDTA and citric acid effectively restored the oxidative stability of the randomized corn oil, but an adsorptive bleaching treatment made the prooxidant problem even worse.

These results suggest that the prooxidant formed during randomization may facilitate the catalytic activity of transition metals in oil oxidation. Therefore, precautions should be taken when using interesterification or randomization processes in the food industry.

Purification may be needed to remove the prooxidant and metal ions, or EDTA or citric acid could be added to prevent the prooxidant activity.

INTRODUCTION

It has been well established that the oxidation rate of triacylglycerol (TAG) is positively correlated to its degree of unsaturation, but studies on the effect of positional change of unsaturated fatty acids in TAG on its oxidative stability have given controversial results (Zalewski and Gaddis, 1967; Fatemi and Hammond, 1980; Kim et al., 1988; Miyashita and Takagi, 1988; Neff et al., 1992).

Raghuveer and Hammond (1967) reported that the rate of autoxidation of mixtures of triunsaturated glyceride (0.5 – 1.5%) and tridecanoin was dramatically decreased after randomization. They also proposed that the concentration of unsaturated fatty acid at the *sn*-2 position of glycerol should stabilize a fat toward oxidation. Later studies confirmed the effect of randomization in stabilizing mixtures of trisaturated and unsaturated TAG towards oxidation at 50°C (Wada and Koizumi, 1983) and also the protection of unsaturated fatty acids at the *sn*-2 position in oxidizing refined soybean oil under high temperature (180°C) treatment (Yoshida and Alexander 1984). However, others showed that there was no preferential oxidation between the *sn*-1(3) and *sn*-2 positions in trilinolein (Neff et al., 1990). Hoffmann et al. (1973) studied the oxidation of a variety of synthetic TAGs at 85°C and suggested that 1,3-equiacyl TAG tended to be more stable toward oxidation than its 1,2-equiacyl isomer.

Research by Park et al. (1983a) showed that there was no difference in the rates of autoxidation before and after interesterification of synthesized TAG mixtures or TAG purified from soybean oil. The increased oxidation rate of randomized TAG was due to the depletion of tocopherols by a purification step after the randomization reaction. They also

found no difference in the oxidation rate when unsaturated acyl groups were present at different positions in TAGs (PLP vs. PPL, PLnP vs. PPLn, where P = palmitate, L = linoleate, Ln = linolenate) (Park et al., 1983c).

Lau et al. (1982) reported that the oxidation rate of corn oil increased three to four times after randomization when tocopherols were removed by alumina column chromatography. They analyzed the composition of oxidized oil and found about three times more scission products in the randomized corn oil.

The present study originally was conducted to confirm the effect on corn oil oxidation of structural change of TAG caused by randomization and to study how randomization changed the oxidation mechanism. However, we found that the randomization of corn oil TAG did not affect their oxidation rate. Rather, randomization can produce a prooxidant whose activity depends on the presence of transition metals in the oil.

LITERATURE REVIEW

Mechanism of Lipid Autoxidation

Autoxidation is a chemical reaction between molecular oxygen and organic compounds. It can take place at ambient temperatures. Autoxidation mainly happens to unsaturated compounds and can result in deterioration and degradation in food, biological tissues and polymers. The process of autoxidation follows a free radical (a free radical is a molecule with an unshared electron) chain mechanism and can be divided into three steps: initiation, propagation and termination (Chan, 1987; Frankel, 1998).

Initiation: $RH + X\cdot \rightarrow R\cdot + XH$

Propagation: $R\cdot + O_2 \rightarrow ROO\cdot$

$ROO\cdot + RH \rightarrow ROOH + R\cdot$

Termination: $ROO\cdot + ROO\cdot \rightarrow$ stable compounds

$ROO\cdot + R\cdot \rightarrow$ stable compounds

$R\cdot + R\cdot \rightarrow$ stable compounds

In the initiation step, $X\cdot$ is an initiator, which can abstract hydrogen from unsaturated lipid (RH) to from lipid radical ($R\cdot$). $X\cdot$ may be a transition element ion, a radical generated by photolysis or high-energy irradiation, or a radical formed by decomposition of existing hydroperoxide (ROOH). Transition metals (M^{n+} or M^{n+1}) are believed to play an important role in the initiation process, and it is very difficult to completely remove traces of these metals from lipids. The metal-catalyzed decomposition of preformed hydroperoxide becomes the most likely process in the initiation step as indicated below:

$$ROOH + M^{n+} \rightarrow RO\cdot + OH^- + M^{n+1}$$

$ROOH + M^{n+1} \rightarrow ROO\cdot + H^{+} + M^{n+}$

Once the unsaturated alkyl radical (R·) is formed, it reacts very rapidly with molecular oxygen to form peroxy radicals (ROO·) that then react with lipids (RH) to form hydroperoxides (ROOH). An alkyl radical is regenerated in the propagation step, and it can react again with oxygen and form a cycle. As the reaction repeats in this way, more and more hydroperoxides are created. Hydroperoxides are the primary products of lipid oxidation and can be decomposed easily to initiate more free radical chains so the rate of oxidation accelerates with time. The free radicals can decompose and generate smaller molecules, such as ketones, aldehydes, alcohols and acids. These volatile compounds are the major causes for the off-flavors produced during deterioration of food lipids.

In the termination step, two radicals react with each other to form non-radical products and the free radical chain stops. This reaction is important in polymer formation and accelerates when there are more free radicals. An antioxidant can also react with a free radical to produce a stable compound and terminate the free radical chain reaction.

Chemical Interesterification and Its Mechanism

Interesterification is an ester interchange that can be applied to fats and oils. In TAG, it allows acyl group to transfer from position to position on glycerol and between glycerol molecules. Along with hydrogenation and fractionation, interesterification has been used as an important lipid modification method. Interesterification can effectively alter fatty acid distribution in TAG and thus change the physical and functional properties of lipids, such as melting point, solidification behavior and crystal formation. Such manipulation gives the food industry liberty to make plastic fats with proper spreadibility and melting profile, e.g.

shortening, margarine, confectionary fats or nutritional margarine blends with highly polyunsaturated content and zero-*trans* fatty acids (Sreenivasan, 1978).

There are three types of interesterification: a) interchange between a fat and free fatty acids, also called acidolysis, such as the reaction to incorporate acids with low molecular weight into a fat with higher molecular weight fatty acids; b) interchange between a fat and an alcohol, also called alcoholysis, such as the reaction of TAG with methanol to produce fatty acid methyl esters; c) rearrangement of fatty acyl groups between two different fats, also called transesterification (Hustedt, 1976).

Interesterification may proceed in two ways: random and directed. The random interesterification (also known as randomization) will lead to a complete random distribution of fatty acids among TAGs, which means the distribution of fatty acids is determined only by chance. Directed interesterification is carried out at a temperature below the melting point of TAG with the highest melting point (usually the trisaturated TAG) so that it will crystallize as it is formed. As the reaction proceeds, the equilibrium of the reaction will be pushed to produce more high-melting TAGs (Rozenaal, 1992).

Two types of catalysts can be used in interesterification: enzymatic and chemical. Enzymatic modification relies on the use of random or regiospecific (1,3- or 2- specific) and fatty-acid-specific lipase as catalysts. Enzymatic interesterification is particularly useful in making cocoa butter analogue with saturated fatty acids at the *sn*-1,3 position and oleic acid at the *sn*-2 position (Macrae, 1983). In chemical interesterification, acid or base catalysts are employed; among them, metal alcoholates are the most commonly used (Marangoni and Rousseau, 1995). Random chemical interesterification usually results in random distribution of fatty acid in TAG. However, regioselestivity can be accomplished by directed chemical

interesterification using sodium methoxide at low temperature (Knoish et al., 1993). The observed ester interchange at the 1,3- positions was 1.7 times faster than at the 2- position of TAG at 30°C.

Two proposed mechanisms for chemical interesterification can be found in the literature: the carbonyl addition mechanism (Coenen, 1974) and the enolate ion formation (Claisen condensation) mechanism (Weiss, 1961). In the carbonyl addition mechanism (Fig. 1), an alkaline catalyst, such as sodium methoxide that is a strong nucleophilic reagent, can attack the carbonyl carbon of a glycerol ester to produce a glycerylate anion and methyl ester. The glycerylate anion reacts with another glyceride by nucleophilic attack of its carbonyl carbon, abstracting a fatty acid, thus forming a new TAG. A new glycerylate ion is also formed and can react with other TAGs. In the Claisen condensation mechanism (Fig. 2), the sodium methoxide abstracts an acidic hydrogen from the α-carbon of the glycerol ester, forming an enolate ion, which is a strong nucleophile and will attack carbonyl groups to form a β-keto ester and a glycerylate ion. The glycerylate ion can attack other carbonyl groups and exchange the acyl groups among glycerol esters.

It should be noted that both these mechanisms are speculative pathways that lack solid experimental support. Evidence for the formation of β-keto ester in the Claisen condensation mechanism is the presence of an infrared absorption peak at 6.4 μm in the interesterified oil. However, this absorption is also found with ionized carboxyl groups of soap, which is a common by-product in the chemical interesterification (Bellamy, 1975). In a recent research, Liu (2004) used different esters of carboxylic acids as substrates in methoxide-catalyzed interesterification and found that the esters of α-methyl-substituted

acids did not participate in the ester-ester interchange. This observation provided some supportive evidence for the α-proton abstraction during enolate ion formation.

Effect of Triacylglycerol Composition and Structure on Lipid Oxidation

The factors that may affect the TAG oxidation rate include the overall unsaturation of the TAG (type of TAG), the unsaturation of acyl groups in the TAG (number of double bonds), the position of unsaturated fatty acid in the TAG and also the physical status of the oil, e.g. its viscosity or surface to volume ratio (Miyashita and Takagi, 1988; Knothe and Dunn, 2003). Therefore, the final oxidation rate of TAG could result from a combination of effects of all these factors.

The oxidation rates of fatty esters generally increase with the number of double bonds in a molecule. The relative rates for linolenic, linoleic and oleic acid methyl esters are approximately 20:10:1 (Gunstone and Hilditch, 1946). Small amounts of more highly unsaturated fatty compounds containing bis-allylic carbons (such as in linoleate and linolenate) have a disproportionately strong effect on oxidative stability (Gunstone and Hilditch, 1946; Knothe and Dunn 2003).

The effect of TAG structure, especially the position of unsaturated fatty acid on its oxidative stability has been studied extensively in the past 40 years, but the results are still controversial. Raghuveer and Hammond (1967) first reported that the rate of autoxidation of mixtures of triunsaturated glyceride (0.5 – 1.5%) and tridecanoin was dramatically decreased after randomization. They also found that the randomization of natural fats increased their oxidation and proposed a theory that the concentration of unsaturated acyl groups at the *sn*-2 position of glycerol increased the oxidative stability of resulting TAG. The same study also

predicted that when the proportion of unsaturated acyl groups in the TAG increased, the difference in the oxidation rate before and after randomization should decrease. Similarly, Wada and Koizumi (1983) reported that the randomized mixtures of tri-saturated and unsaturated TAG (tripalmitin, tristearin, triolein and trilinolein) were much more stable than their original mixtures during oxidation at 50°C. They also analyzed the remaining TAGs at different stages of oxidation and concluded that TAGs having unsaturated fatty acid linked at the *sn*-2 position were more stable towards oxidation than those linked at the sn-1, 3 position.

Lau et al. (1982) showed a three to four fold increase in the oxidation rate of corn oil after randomization when the natural and randomized oils were treated with alumina to remove tocopherols. They analyzed the composition of oxidized oil and found about three times more scission product in the randomized corn oil and suggested the TAG structure may affect the rate of initiation.

In a study to examine the changes in the structure of soybean TAGs during heat treatment (180°C for 50-100 h) with aeration, Yoshida and Alexander (1984) found that the percentage of the fatty acids remaining in the 2-position of the residual TAGs remained nearly unchanged throughout heating. Their results indicate that unsaturated fatty acids located at *sn*-2 position of TAG are protected significantly from thermal oxidative decomposition.

Neff et al. (1994) studied the oxidative stability of blends and interesterified blends of soybean and palm TAGs. The oxidation was carried out at 50°C in the dark and the peroxide value (PV) and oxidation products were measured. They reported that the blends were more stable towards oxidation before than after they had been interesterified. The analysis of fatty acid composition at *sn*-2 and *sn*-1,3 of TAG showed that interesterification increased the

percent of oleic acid and linoleic acid at *sn*-1,3 position and decreased their percent at the *sn*-2 position, which may be part of the reason for the decreased stability of the interesterified TAG. In another report by Konishi et al. (1995), the oxidative stability of regioselective chemical interesterification (catalyzed by sodium methoxide at 30°C) product of soybean oil and methyl stearate was compared with soybean TAG and the random intereseterification product. They found that the regioselective interesterification produced a sample with the lowest peroxide formation and concentrations of volatile oxidation products. The fatty acid composition and positional distribution on the glycerol backbone of the TAG showed that the regioselective interesterification resulted in about 10% more linoleic acid at *sn*-2 position than that in the product produced by random interesterification.

Hoffmann et al. (1973) examined a variety of synthetic TAGs and their oxidation rates as measured by their consumption of oxygen at 85°C. They concluded that 1,3-equiacyl TAG tended to be more stable to oxidation than its 1,2-equiacyl isomer. For example, 1,3-dioleoyl-2-linoleoylglycerol (OLO) was much more stable than 1,2-dioleoyl-3-linoleoylglycerol (OOL), and 1,3-distearoyl-2-oleoylglycerol (SOS) was much more stable than 1,2-distearoyl-3-oleoylglycerol (SSO). They also noticed the striking instability of SSO compared to SOS, OOO and OSO, which could not be explained very well by the degree of unsaturation and protection of unsaturated fatty acid at *sn*-2 position.

By measuring peroxide value (PV) at 50°C, Miyashita and Takagi (1988) reported that the autoxidation rates of safflower oil esters increased with increasing numbers of acyl groups per molecule in the following order: monoacylglycerol (MAG), methyl ester (ME), diacylglycerol (DAG), TAG and sucrose ester (SE). They explained this by the idea that the intramolecular free radical chain reaction between acyl groups of esters occurs more rapidly

than an intermolecular chain reaction. They also found that 1,3-dilinolein oxidized faster than 1,2-dilinolein, suggesting that acyl groups may have less contact with each other in 1,3-dilinolein than in 1,2-dilinolein.

By using synthetic TAG, Neff and El-Agaimy (1996) showed that 1,3-dipalmitoyl-2-linoleoyl-glycerol (PLP) oxidized faster than 1,2-dipalmitoyl-3-linoleoyl-glycerol (PPL) at 60°C based on PV. 1-palmitoyl-2,3-dilinoleoyl-glycerol (PLL) oxidized faster than 2-palmitoyl-1,3-dilinoleoyl-glycerol (LPL). They also proposed that interaction between adjacent unsaturated fatty acids in TAG facilitated their oxidation.

Miyashita et al. (1990) studied the autoxidation of four synthetic TAGs containing linoleic acid (L) and linolenic acid (Ln) at 40°C and found Ln oxidized twice as quickly as the L components. LnLnL oxidized slightly faster than LnLLn in terms of induction periods (45 vs. 47 hr). LLnL oxidized a little faster than LLLn (56 vs. 60 hr). However, these differences were not very great. Neff et al. (1990) reported that no preferential oxidation occurred between the *sn*-1(3) and *sn*-2 positions in trilinoleoylglycerol at 40°C. Probably, as the degree of unsaturation of TAG becomes as great as five double bonds or more, the oxidative stability may not be affected greatly by an acyl group's position.

In a study on the oxidative stability of oils from selected soybean germplasm at 60°C, Neff et al. (1992) analyzed the correlation coefficients between peroxide value or headspace volatiles and the fatty acid composition and acyl group distribution on the glycerol. They found that the rate of peroxide formation was positively correlated with average number of double bonds ($r = 0.81$), linoleic acid ($r = 0.63$) and linolenic acid ($r = 0.85$), and negatively correlated with oleic acid ($r = -0.82$). They also observed a positive correlation between the peroxide formation and linoleic acid ($r = 0.72$) at the *sn*-2 position of the glycerol.

However, not all studies have shown different oxidation rates because of TAG structure change, such as those caused by randomization. Zalewski and Gaddis (1967) reported that there was no difference in the oxidation rate of natural and randomized lard at 60°C after removing tocopherols by a molecular distillation and concluded that the changes in stability could be attributed to the decomposition of tocopherols. They argued that the higher rate of oxidation of the triunsaturated and saturated TAG blends compared with their randomized products reported by Raghuveer and Hammond (1967) might be merely due to the highly susceptible TAG type (triunsaturated) in the blends, instead of fatty acid positional changes caused by randomization. Hoffmann et al. (1973) tried to offer an explanation for such discrepancy. They found that randomized palm TAG oxidized much more rapidly than natural palm TAG at 85°C, whereas the oxidation rate of lard TAG was nearly unchanged after randomization. They attributed this to a slight change after randomization of the SSO (where S = saturates, O = oleate) content in lard (from 25 to 19%) as compared with a considerable change of SSO content in palm oil (from 7 to 20%), because SSO was found strikingly unstable.

A study conducted by Kim et al. (1988) showed almost no difference between the oxidation of mixed soybean:palm oil (1:1) or soybean:coconut oil (1:1) and their interesterified products during continuous heating at 180°C for 50 h. The methods used to evaluate the oxidative stability were PV, carbonyl value (CV), acid value (AV) and refractive index (RI). However, since peroxides can be decomposed easily at high temperatures, it is not appropriate to use PV in such a study. The RI of interesterified oils increased more rapidly than that of the blend.

Research by Park et al. (1983 a, b, c) also found no difference in the oxidation rates of TAG caused by positional changes of acyl groups. Their experiments showed there was no difference in the rates of autoxidation before and after interesterification of synthesized TAG mixtures or TAG purified from soybean oil. Also, no difference was observed when TAGs with unsaturated acyl groups present at different positions (PLP v.s PPL, PLnP v.s. PPLn) were oxidized (Park et al. 1983a, c). They used both PV and rate of consumption of dissolved oxygen at 37 or 50°C as the measurements for lipid oxidation rate. They further demonstrated that the increase of the oxidation rate of randomized TAG was due to the depletion of tocopherols by the purification step used after the randomization to remove soaps or colored substances (Park et al. 1983a). These results suggest that experimental conditions, especially oil purification, should be controlled when studying effect of randomization of TAG on its oxidative stability. In another study with soybean TAG in similar condition, they concluded that the oxidation rate of each TAG molecular species increased with the higher degree of unsaturation and the longer saturated acyl chain present in these TAGs (Park et al. 1983 b).

A recent study (Hoshina et al., 2004) on the thermal oxidative stability of edible oils showed that a 2:1 (mol/mol) mixture of the trisaturated TAG PPP (where P = palmitate) and triunsaturated TAG (LLL, where L = Linoleate) oxidized faster than PPP/PLL (1:1) and PPL at 180 or 150°C. Similarly, a 2:1 mixture of PPP and OOO or LnLnLn (where O = oleate, Ln = linoleneate) oxidized faster than PPO or PPLn, respectively. They concluded that the thermal oxidative stability of TAG is negatively correlated with the moles of unsaturated fatty acid in an individual TAG molecule. Their results agree with findings by Endo et al. (1997) who evaluated the oxidation of TAG containing eicospentaenoic acid at 25°C.

Generally speaking, the oxidative stability of TAG can be greatly affected by altering the number of unsaturated fatty acid in individual TAG, degree of unsaturation of fatty acid and possibly the position of unsaturated fatty acid on glycerol. A general rule for the effect of positional change of unsaturated fatty acid on the oxidation rate of TAG has not been formulated. However, it seems that, with a high degree of unsaturation of TAG, the effect of unsaturated fatty acid position on the TAG oxidation is not very great. Also, the experimental condition and evaluation methods for oxidation may affect the results in such studies.

MATERIALS AND METHODS

Two brands of corn oils (Elite, Bunge Foods, Bradley, IL; and Vital, ACH Food Companies, Inc., Memphis, TN) were purchased locally. Palmitic acid, 1-monosteroyl-*rac*-glycerol (99% purity), 1-monolinoleonyl-*rac*-glycerol (99% purity) and pancreatic lipase were from Sigma (St. Louis, MO). Dipalmitin was from Nu-chek-Prep (Elysian, MN). Sodium methoxide, adsorptive alumina, calcium chloride, tris(hydroxymethyl)methylamine, ammonium thiocyanate, hydrated barium chloride, hydrated ferrous sulfate, hydrochloric acid, sodium sulfate, ferric ammonium sulfate and cupric acetate were from Fisher Scientific (Pittsburgh, PA). All solvents, including hexane, diethyl ether, ethanol, benzene, methanol and ethyl acetate (HPLC grade) were purchased from Fisher Scientific. Acetyl acetone, ethyl acetoacetate and ethyl diacetoacetate were from Sigma-Aldrich.

Oil randomization. Corn oil was randomized with 0.5% sodium methoxide at 60-80°C for 1 h under vacuum. Acetic acid was added to terminate the reaction, and the oil was washed with warm water (35-40°C) until neutral. Sodium sulfate was stirred with the oil to absorb residual water. Centrifugation at 1431 *X g* for 5 min (IEC CentraMP4, International Equipment Company, Needham Heights, MA) was applied to obtain clear oil.

Confirmation of randomization by pancreatic lipase hydrolysis assay using the method of Luddy et al.(1967). 50 mg of oil was weighed into a 5-ml screw-cap vial with ~9 mg pancreatic lipase. Then 1.0 mL of 1 M tris(hydroxymethyl)methylamine buffer (adjusted to pH 8.0) and 0.1 mL of 22% calcium chloride solution were added. The vial and its contents were warmed to 40°C for 1 min and then mixed with a Vortex mixer (Fisher vortex Genie 2, Fisher Scientific, Pittsburgh, PA) at the highest rate for ~1 min. The mixture was

extracted three times with 2 ml of diethyl ether and the combined ether layers were washed with distilled water, dried over sodium sulfate and the solvent evaporated. The extracted lipid was separated with a preparative thin-layer chromatography (TLC) plate (Adsorbosil plus 1, 20 X 20 cm, 500 μm, Alltech, Deerfield, IL) using hexane:diethyl ether:acetic acid (60:40:2; v/v/v). The 2-monoacylglycerol band was collected and transesterified on the silica by adding 2 mL of 1 N methanolic sodium methoxide and reacting for 1 h at room temperature. Then hexane was added to extract the fatty acid methyl esters, which were then injected into a capillary column (Supelco SP-2330, 15m X 0.25mm, 0.2 μm,) for GC analysis in an Agilent 5890 Series II Instrument (Agilent Technologies, Palo Alto, CA) with oven temperature at 190°C, detector and injector temperature at 230°C and carrier gas of helium at 1 mL/sec.

Oil purification. Oil dissolved in hexane was passed through an alumina column to remove impurities and antioxidants according to the method of Jensen (1966). Alumina was activated by heating to 260°C overnight. The oil was passed through an alumina column using a 1:2 ratio of oil to alumina. The oil was eluted with three column volumes of 5% diethyl ether in hexane. Adsorbed material was recovered by eluting with three column volumes of methanol. When needed, the column extract was further concentrated by two alumina purification steps to obtain a highly concentrated fraction for TLC separation, which was carried out under the same conditions as the 2-monoacylglycerol separation.

Oxidative stability test. Five grams of oil sample in a 125-mL Erlenmeyer flask was oxidized at 28, 40, or 50°C in an incubator in the dark depending on the experimental design. Oil samples were taken periodically and the peroxide value (PV) was measured using the ferrous iron oxidation method (Driver et al., 1963). An oil sample of 50-400 mg was weighed

accurately into a 10-ml volumetric flask and diluted to the mark with ethanol-benzene (80:20). Next, 50 μL ammonium thiocyanate solution (30 g/100 mL = 3.75M) and 100 μL ferrous chloride solution (approx. 0.014 M) were added to the flask and mixed well. After 10 min, the absorbance was measured at 515 nm with a Hitachi U-2000 spectrophotometer (Hitachi Instruments, Inc., Tokyo, Japan) and the PV was calculated based on a standard curve. The standard curve was made by plotting the absorbance of dilutions of an oxidized oil sample against the micro equivalents of peroxide calculated from the oil's PV as determined by the official AOCS method Cd 8-53. The oxidation rate constant was determined from the slope of the trend line (linear regression) of natural logarithm of PV versus time (Uri, 1961).

Oil Stability Index (OSI) test. Oil stability was tested in an ADM OSI (Oxidative Stability Instrument, Omnion, Rockland, MA) instrument at various temperatures.

Analysis of free fatty acid. Free fatty acid (FFA) in oil was determined by titrating with sodium hydroxide according to official AOCS method Ca 5a-40.

Determination of fatty acid composition of oil. About 50 mg of oil was transesterified with 2 mL of 1 N methanolic sodium methoxide for 1 h at room temperature. The reaction was terminated by adding 1 mL water and the fatty acid methyl esters were extracted by hexane for GC analysis as previously described.

Quantification of monoacylglycerol (MAG) and diacylglycerol (DAG) in corn oil. MAG and DAG contents of corn oil were analyzed by high performance liquid chromatography (HPLC) according to the method of Liu et al. (1993). An oil sample was dissolved in hexane/2-propanol (90/10; v/v) at a concentration of about 20 mg/mL, and 20 μL of the solution was injected into a Beckman Coulter HPLC with System Gold

autosampler 508 and solvent delivery module 126 (Fullerton, CA). An evaporative light-scattering detector (Alltech ELSD 2000, Deerfield, IL) was used with the drift tube temperature set at 55°C and carrier gas (nitrogen) flow rate at 1.6 L/min. A Chromegasphere SI-60 column (150 x 3.2 mm, 5 μ, ES Industries, Marlton, NJ) was used for separation. The mobile phase was: channel A, hexane; channel B, hexane/2-propanol/ethyl acetate/10% formic acid in 2-propanol (80/10/10/1; v/v/v/v). The mobile phase gradient program is shown in Table 1, and the flow rate was 0.7 mL/min. 1,3-DAG was well separated from 1,2-DAG and 1-MAG was separated from 2-MAG by this method. 1,3-Dilinolein and 1-monolinolein (Nu-Chek-Prep, Elysian, MN) with 99%+ purity were used in making standard curves. The log (response) vs. log (mass) was linear for MAG, but not for DAG, which is not in agreement with the observation of Liu et al. (1993). This is probably due to a higher amount of DAG (up to 42 μg) that was used for calibration in our study. Therefore, all the MAG and DAG contents were calculated based on the standard curve of response vs. mass, instead of log (response) vs. log (mass).

Synthesis of MAG and DAG from corn oil by a chemical method. Corn oil and methanol was mixed at the molar ratio of 1:2 and transesterified by adding 2% sodium methoxide and stirring overnight at room temperature. After acetic acid was added to terminate the reaction, the oil was washed by distilled water for 5 times and dried over anhydrous sodium sulfate. The transesterified oil was passed through an alumina column in a ratio of 1:2 (oil:alumina). The neutral oil was washed out by two column volumes of 10% ether/hexane, the MAG and DAG fraction was eluted by three column volumes of methanol and the solvent was then evaporated. The synthesized MAG and DAG were separated by preparative TLC and extracted with methanol as described previously.

HPLC analysis of tocopherol. Tocopherols were quantified with a 250 mm, 7 μ Lichrosorb Si 60 column (E Merck Darmstadt, F.R Germany) in a Beckman Coulter HPLC equipped a System Gold 168 detector (Fullerton, CA) at 292 nm. The solvent was hexane:isopropanol (99.25:0.75; v/v). The flow rate was 0.5 mL/min for 0 – 7 min, then programmed to 0.7 mL/min from 7 – 7.3 min, held for 7.5 min, and decreased to 0.5 ml/min from 14.8 – 15 min.

GC/MS characterization of MAG extract from TLC. Column extract eluted by methanol from the alumina used to purify randomized oil was separated by TLC as previously described. The MAG band was collected and extracted with methanol. The MAG extract was analyzed by GC-MS using a Micromass GCT mass spectrometer (Micromass, Beverly, MA). The sample was separated by a DB-5 MS column (30m X 0.25mm, 0.25 μm, J&W Scientific, Folsom, CA) in a Agilent 6890 Series GC System (Agilent Technologies, Palo Alto, CA) with oven temperature programmed as 150 °C (1min) – 15° C/min – 300 °C (5min), injector temperature at 260 °C, carrier gas of helium at 1mL/sec, split ratio of 25/1. The MS condition was electron impact (EI) ionization with scan cycle at 0.75 second and mass range from 45 to 500.

Bleaching treatment of oil. Bleaching earth (acid-activated) at 2% was mixed with oil and heated at 100°C in a rotary evaporator under vacuum for 20 min. The oil was then filtered through a filter paper (Qualitative #2, 90 mm diameter x 100 circles, Whatman Inc., Clifton, NJ) to obtain clear oil.

"Randomization" of triacetin. Triacetin was washed three times with an equal amount of distilled water. The water-washed triacetin was dried over anhydrous sodium sulfate and then "randomized" with 0.5% sodium methoxide at 60-80°C for 1 h under

vacuum. After the reaction, carbon dioxide gas was sparged through the randomized triacetin for 1.5 h to inactivate the catalyst and the catalyst was removed by filtration.

GC-MS analysis of "randomized" triacetin. "Randomized" triacetin dissolved in methanol was separated by a DB-5 MS column (30 m X 0.25 mm, 0.25 μm, J&W Scientific, Folsom, CA) in an Agilent 6890 Series GC System (Agilent Technologies, Palo Alto, CA) with oven temperature programmed from 100 °C to 250 °C at the rate of 10° C/min, injector temperature at 260 °C, carrier gas of helium at 1mL/sec, split ratio of 25/1. The sample was analyzed in a Micromass GCT mass spectrometer (Micromass, Beverly, MA) using chemical ionization by ammonium, with scan cycle at 0.75 sec and mass range from 80-700.

Statistical analysis. All statistical analyses were performed by using PC SAS (SAS Institute, Cary, NC, USA) software using a general linear module (GLM).

RESULTS AND DISCUSSION

Effect of randomization on oil oxidation before and after purification. The fatty acid composition of natural corn oil and at the *sn*-2 position of natural and randomized corn oil is shown in Table 2. The *sn*-2 fatty acid composition of randomized corn oil was similar to the fatty acid composition of natural corn oil, indicating that the oil was completely randomized after the reaction. The natural or randomized oil was purified through an alumina column to remove antioxidants (mainly tocopherols) and other polar components, such as MAG, DAG and free fatty acid. The fatty acid composition of corn oil was not changed by the purification treatment (data not shown). After purification, both natural and randomized corn oils were oxidized at 28 °C in duplicate in the dark, and oil samples were taken periodically to determine PV. The slope of natural logarithm (Ln) of the PV versus time was determined as the oxidation rate constant (Uri, 1961).

Figure 3 shows the plot of PV versus time of purified natural and randomized corn oil oxidized at 28 °C. It shows clearly that the natural and randomized oil oxidized at very similar rates. The oxidation rates, determined from plots of Ln (PV) vs. time, were 0.903 ± 0.01 and 0.912 ± 0.06 for purified natural corn oil and purified randomized corn oil, respectively. To further confirm such findings, multiple experiments with different lots of corn oils were conducted and the results are summarized in Table 3. Although there were some considerable variations among different lots of oils, statistical analysis showed that there was no significant difference between the oxidation rates of natural and randomized corn oils after purification ($P < 0.05$). Similar results were also obtained by OSI test. The OSI time (h at 60 °C) of natural and randomized corn oil after purification was 15.24 ± 0.98 and

15.42 ± 0.73, respectively. Such results indicate that the TAG structure alteration caused by randomization did not affect the oxidation rate of corn oil TAG.

However, without purification, the randomized oil oxidized much faster than natural oil in the OSI test at 100 °C as shown in Table 4. Various lots of oils had different OSI time, especially after randomization. All the lots of natural corn oils had similar OSI times of about 20 h at 100 °C, but after randomization, the OSI times were much shorter, ranging from 3.60 to 10.67 h. Such differences among lots of oils may be caused by contents of some minor components in the oil, such as tocopherols, iron and copper.

Figure 4 shows that in an OSI test at 80 °C, randomized corn oil oxidized faster than natural oil before alumina column purification. After purification they oxidized at the same rate. Moreover, when the methanol eluate from alumina column used to purify the randomized oil was evaporated (free of the methanol) and added back to the purified randomized oil, it fully restored the OSI time to the same value as the unpurified randomized oil.

These results suggest that some prooxidant is produced during the randomization reaction that can be removed by alumina column purification. Lau et al. (1982) reported that randomized corn oil oxidized three to four times faster than natural corn oil after purification, which was not seen in the current study. It is likely that we did a more complete purification removing all the prooxidants. Although many articles have suggested that the positional change of unsaturated fatty acid in TAG may have some effect on its oxidation (Hoffmann et al., 1973; Wada and Koizumi, 1983; Yoshida and Alexander, 1984; Miyashita and Takagi, 1988; Neff et al., 1992), no difference was shown between natural and randomized corn oil TAG in our study. This maybe due to the polyunstaturated nature of corn oil TAG, which

makes such an effect not significant. Park et al. (1983a) also found no difference in the oxidation rates of natural and randomized TAG from soybean oil after purification.

Test on possible prooxidants: free fatty acids and their sodium salts. Free fatty acids can be prooxidants in natural or purified soybean oil (Miyashita and Toru Takagi, 1986; Mistry and Min, 1987; Yoshida et al., 1992). We found that the free fatty acid contents in natural and randomized corn oils were 0.06% and 0.6%, respectively. To test the effect of free fatty acids on oil oxidation, 0.6% palmitic acid was added to natural corn oil and oxidized at 100 °C by the OSI method. The corn oil with free fatty acid had an OSI time of 18.35 ± 0.88 (h at 100 °C), which is not statistically different from the OSI time of natural corn oil (18.93 ± 0.46). The OSI time of randomized corn oil was 10.67 ± 0.08. Such results indicate that free fatty acid produced during randomization was not the prooxidant in the randomized corn oil.

Since sodium methoxide was used as the catalyst, sodium fatty acid soap could be formed during the reaction. Acetic acid was added at the end of the reaction, so sodium acetate can be formed also. We suspected that sodium could be a prooxidant in oil, therefore, both sodium oleate and sodium acetate were added to natural corn oil and the blends were tested by OSI. With up to 100 ppm sodium added as in sodium oleate, the OSI time of natural corn oil was reduced from 20.64 (h at 100° C) to 16.63 (Table 5). However, this reduction was not great compared with the OSI time of randomized corn oil (5.68 h). In a separate experiment, 0.6% sodium oleate (equal to 454 ppm sodium) reduced the OSI time of natural corn oil to 15.17 h. Sodium acetate also showed some prooxidant effect in corn oil. About 50 ppm sodium as sodium acetate showed the most significant effect and reduced the OSI time of natural corn oil from 21.30 to 12.91 h. Greater amounts of sodium acetate had less effect.

Further experiments showed that sodium acetate could be easily washed out by water. At the end of the reaction, excess amounts of acetic acid were added to the oil, so the majority of sodium fatty acid soap formed during the reaction should be converted to sodium acetate and free fatty acid. Sodium acetate was then washed out by water. According to our analysis, 0.6% free fatty acid (equal to 21.2 μmol oleic acid/g of oil) was present in the randomized oil, which indicates the maximal possible amount of sodium soap formed during the reaction. After acetic acid addition and water washing, the amount of sodium soap left in oil should be less than 10% of the maximum, which equals to 2.12 μmol sodium/g oil or 49 ppm sodium. The above results indicate that sodium ion (as low as 10 ppm) has some prooxidant effects, but is not likely to account for the prooxidant in the randomized corn oil.

Effect of tocopherol levels on oil oxidation. Our HPLC analysis showed that there was about 10% reduction in the total tocopherol content in the randomized corn oil (α: 170 ppm; β: 620 ppm; γ: 34 ppm; and total: 824 ppm), compared to natural corn oil (α: 222 ppm; β: 658 ppm; γ: 33 ppm; and total: 913 ppm). To test if such a 10% reduction of tocopherols could account for the increased oxidation of randomized oil, different amount of tocopherols were added to purified corn oil and the oil stabilities were tested by the OSI at 100 °C. Table 6 showed that 90% tocopherol added to alumina purified oil did not decrease the OSI time of the oil (14.40 h), compared to 100% tocopherol in the purified oil (14.60 h). When the tocopherol level was decreased to 60%, the OSI time was reduced significantly (13.20 h). However, 100% tocopherols addition in purified corn oil did not fully restore its OSI time to the same level as natural corn oil. We are not sure why this happened. Tocopherols added to unpurified corn oil at 60% and 100% of the natural level increased stability only slightly.

Effect of MAG and DAG on oil oxidation. Since column extract eluted by methanol from the column used to purify randomized corn oil showed a prooxidant effect, the composition of the column extract was studied. The column extract was separated into MAG, DAG and TAG bands on TLC. Each band was scraped off the plate, extracted by methanol, and added back to natural corn oil which was subjected to the OSI test. Some prooxidant effect was associated with the MAG and DAG bands, but not with the TAG band (Table 7). The MAG band appeared to have more prooxidant effect than the DAG band, although they were not different statistically. The silica between each band on the TLC plate was also collected and tested, but showed no prooxidant effect.

HPLC quantification showed there was an increase of MAG and DAG contents of corn oil after randomization. Analysis of two types of corn oil showed that before randomization, there was no MAG detected and about 1.4% DAG in the oil. After randomization, the MAG content increased to 0.2 – 0.4% and DAG content was about 5%. After the randomized oil was purified by alumina column, no MAG or DAG was detected.

With such information, the effect of linoleate MAG and DAG on oil oxidation was studied. Various amounts of MAG and DAG were added to purified corn oil and it was oxidized at 28 °C. PVs were determined and oxidation rates were calculated. When the MAG level was higher than 0.25% and the DAG level was higher than 5%, there was an increase of oxidation rate of purified corn oil (Fig. 5). However, the percentage increase was only about 15% of the oxidation rate of the purified corn oil. Such a limited increase in oxidation rate in purified oil did not seem to account for the greatly increased oxidation rate of unpurified randomized oil, especially considering the unpurified oil contained great amounts of tocopherols.

An OSI test was then conducted by adding various amounts of linoleate MAG to natural corn oil and oxidizing it at 100 °C. These tests showed that no increase of oxidation with MAG at levels up to 0.5% (Table 8). Such results suggest that MAG and DAG are not the prooxidants chiefly responsible for the increased oxidation rate of unpurified randomized corn oil, but the prooxidant seems to have a polarity similar to MAG and DAG when separated on TLC plate.

Experiments with saturated MAG (1-monostearoly-glycerol) and DAG (1,3-dipalmitoyl-glycerol) showed that saturated MAG significantly lowered the OSI time of natural corn oil at the levels of 1 - 2%, but DAG did not show any prooxidant effect at the levels up to 1% (Table 9).

Mistry and Min (1988) reported that 0.25 – 0.5 % stearate or linoleate MAG and DAG showed a prooxidant effect in silica column purified soybean oil at 55°C as measured by headspace oxygen. In another experiment, Chung et al. (2004) also reported 1% oleate MAG had a prooxidant effect in unpurified soybean oil. These results with MAG are consistent with our findings in corn oil, but we did not find any pooxidant effect of linoleate DAG in purified corn oil at levels up to 1.5%.

MAG and DAG synthesized from corn oil were tested by OSI. MAG and DAG were synthesized from corn oil by both chemical and enzymatic methods, separated on preparative TLC plates and then extracted with methanol. In the chemical method, corn oil was mixed with methanol in the molar ratio of 1:2, and 2% (relative to total mixture) sodium methoxide added as catalyst. The reaction was carried out at room temperature overnight and terminated by adding acetic acid. The yields of MAG and DAG by this method were very low. The enzymatic method was similar to the pancreatic lipase hydrolysis assay (Luddy et al., 1967).

The yields of MAG and DAG from enzymatic hydrolysis were higher than from the chemical method. DAG at 0.6% addition (OSI time 17.95 – 18.90 h at 100 °C) did not show considerable prooxidant effect on natural corn oil (19.58 h). However, at the level of 0.6%, MAG synthesized from both chemical and enzymatic methods greatly lowered the OSI time of natural corn oil to 9.15 h, which was similar to that of randomized oil (10.60 h). Such results suggest that the prooxidant may be produced during both chemical and enzymatic randomization reaction. However, the experiment was not repeated.

GC-MS analysis of column extract. To further exam what may be present in the MAG band separated by alumina column chromatography and TLC from randomized corn oil, GC-MS analysis was conducted. The major components were oleate and linoleate MAGs and methyl esters. However, no compounds with unusual structures were identified. There were a few very small peaks that could not be identified.

The prooxidant effect of methanol eluate from the alumina column. In later experiments the methanol extract from the alumina treatments of both natural and randomized corn oil showed prooxidant effects in the OSI test (Table 10). Further experiment showed that the methanol eluate from alumina not exposed to corn oil had a slight prooxidant effect (OSI of 16.18 h at 100 °C), but the 5% ether/hexane eluate did not (21.13h). This suggests that there may be some polar impurities in the alumina that could act as a mild prooxidant. The OSI time of corn oil with methanol eluate from pure alumina (16.18 h) was similar to that of the methanol eluate from alumina used for corn oil purification (15.88 h). The prooxidant effect associated with alumina complicated the recovery and identification of the prooxidant produced by randomization from the column extract.

Effect of "randomized" triacetin on oil oxidation. To examine if a simple TAG such as triacetin could produce a prooxidant on exposure to sodium methoxide, triacetin was "randomized" as described previously. Triacetin and "randomized" triacetin were mixed with corn oil in a 1:1 ratio and tested by OSI at 100 °C. A water extract of triacetin was found to have a prooxidant effect, therefore, the randomization was done with the residual triacetin after water wash. The results are summarized in Table 11. After a water wash, the OSI time of the residual triacetin (Tri-rs1) with corn oil was 29.89 h. The OSI time of corn oil with triacetin was not improved by a second water wash and the second extract of triacetin (Tri-Ex2) added to corn oil had no prooxidant effect. Thus, the prooxidant in the triacetin was removed by the 1st water wash. The corn oil with "randomized" triacetin had an OSI time of 27.75, which was not significantly different from that of corn oil with Tri-rs1 (29.89 h). However, when the water extract (about 1.3 g) from 5 g of "randomized" triacetin was added to corn oil (some Tri-rs1 was added to make up 5 g of triacetin part in total), the OSI time was significantly decreased to 24.83 h, suggesting that some prooxidant may be produced in "randomized" triacetin, but more importantly, the prooxidant may be activated by water.

GC-MS analysis showed that the components in the water extract of randomized triacetin were diacetin, triacetin and diacetin monopropanoate. In randomized triacetin, there were about 1.41% diacetin, 98.1% triacetin and 0.49% diacetin monopropanoate. In initial triacetin, there was about 0.53% diacetin, 98.75% triacetin and 0.69% diacetin monopropanoate. Diacetin and diacetin monopropanoate are probably impurities in this commercial product.

Oxidation of purified and then randomized corn oil. It was surprising to find that the purification of corn oil followed by randomized (Corn-P-R) yielded a product that oxidized

much slower than the randomized and then purified corn oil (R-corn-P) or purified corn oil (Corn-P). Figure 6A shows that at 40 °C, it took almost 6 days for Corn-P-R to reach PV 20, while R-corn-P and Corn-P reached PV 20 in about 1 day. If purified through alumina column again, this oil (Corn-P-R-P) lost its stability and oxidized rapidly (Fig. 6B). HPLC analysis showed that there was about 6.6 – 8 ppm γ–tocopherol present in the Corn-P-R sample, while no tocopherols were detected in R-corn-P and Corn-P. When 6.6 ppm γ–tocopherol was added to Corn-P, this oil oxidized similarly as the Corn-P-R sample (Fig. 6C), which indicated that this small amount of tocopherols can greatly stabilize the purified corn oil.

The small amount of free tocopherols might be released from some tocopherol esters in the purified oil during randomization. Since tocopherol esters are non-polar and have no antioxidant activity theoretically (Gregory, 1996), they could be co-eluted with purified oil without affecting oxidative stability. In order to elucidate whether the tocopherol esters in purified oil were from the natural corn oil or synthesized from free tocopherols and fatty acids during alumina column purification, 600 ppm γ–tocopherol (γ-Toc) was added to purified corn oil in order to match the γ–tocopherol level in natural corn oil and the oil was then purified through alumina column. No free tocopherols were detected in this sample [(Corn-P + Toc)-P]. However, when this sample was randomized, 6.6 ppm γ–tocopherol was detected, which was similar to the tocopherol level in the Corn-P-R sample (6.7 ppm γ–tocopherol). If the alumina treatment indeed could convert free tocopherol to its ester, there should have been more free tocopherols in the [(Corn-P + Toc)-P] sample after randomization as this oil had gone through alumna purification twice. These results suggest that some tocopherol esters present in natural corn oil could be eluted with purified oil and be

released as free tocopherols during randomization. This small amount of free tocopherols could greatly stabilize the purified oil at 40°C.

Effect of EDTA (ethylene diamine tetraacetic acid) and citric acid on oil oxidation. EDTA and citric acid restored the OSI time of unpurified randomized corn oil at 100°C (Fig. 7). A level of 100 ppm EDTA fully restored the OSI time of randomized oil (3.78 h) to 22.43 h, whereas 100 ppm citric acid partially restored the OSI time to 12.83 h. Citric acid at 200 ppm further increased the OSI time of randomized oil to 16.68 h, but still did not reach the same level as natural corn oil (19.85 h). EDTA was more effective than citric acid on inactivating the prooxidant in randomized corn oil. Experiments confirmed that the effect of EDTA was dose dependent, and, at the level of 100 ppm, EDTA could even make the OSI time of randomized corn oil longer than that of natural corn oil (Fig. 8). This result was unexpected, but was repeated in a third experiment in which both EDTA and citric acid were tested at various levels (Fig. 9). EDTA at 100 ppm increased the OSI time of randomized oil from 10.30 h to 25.83 h. Citric acid at 50 ppm fully restored the OSI time of randomized oil to 20.20 h, but its effect was not increased by going to 100 ppm (20.40 h). At the level of 200 ppm, citric acid slightly increased the OSI time of both natural (21.80 h) and randomized oil (21.30 h). Citric acid at 100 ppm was more effective than that in the previous experiment showed in Fig. 7, possibly due to the different OSI time of these two randomized oils (3.78 h vs. 10.30 h).

EDTA and citric acid are both metal ion chelators that are known to make metal ions less effective on catalyzing oxidation. The ability of EDTA and citric acid to increase the stability of randomized oil suggests that the prooxidant produced during randomization may be sensitive to metals. Metal ions are present at very low concentration in fully refined

vegetable oils, with 0.1-0.3 ppm iron and 0.02-0.06 ppm copper (Evans et al., 1974). Therefore, we tested the effect of additional metal ion on the oxidation of randomized oil.

Effect of iron and copper ions on oil oxidation. Small amounts of iron or copper were added to natural and randomized corn oil, and the oil was tested by OSI at 100 °C. The results are presented in Figure 10. Small amount of copper could greatly decrease the OSI time of randomized oil, but not that of the natural corn oil. Iron had a similar effect, but copper seemed more effective than iron. This confirmed that the prooxidant in randomized oil is sensitive to or activated by metal. However, when the natural corn oil with additional iron and copper was randomized, its OSI time (2.88 h) was not as low as that of the randomized oil mixed with iron and copper (1.13 h). Possibly, some of the iron or copper could be washed out by water in the post-randomization process. This result also suggests that metal ions are not directly involved in the formation of the prooxidant during randomization.

Similar results with metal ions were obtained using a PV test at low temperature (Fig. 11). When 0.15 ppm cupric ion was added to unpurified natural and randomized corn oil, and the oil was oxidized at 50 °C, the addition of copper greatly increased the oxidation rate of randomized oil, but not of the natural corn oil. However, the addition of cupric ion (0.06 ppm) did not increase the oxidation rate of either purified natural or purified randomized oil when oxidized at 28°C (Table 12). Thus, alumina column purification effectively removed the prooxidant from the randomized oil.

Effect of copper on the oxidation of purified and then randomized corn oil (Corn-P-R). As shown in an early experiment (Fig. 6), the Corn-P-R oxidized very slowly at low temperature because of the small amount of tocopherol released from its ester in the purified

oil during randomization. To further investigate whether or not the prooxidant was formed in the purified oil during randomization, 0.06 ppm copper was added to the Corn-P-R sample and the oil was oxidized at 40 °C. Corn-P-R oxidized faster with copper added (oxidation rate: 0.996 ± 0.004 with Cu, 0.616 ± 0.012 without Cu), which indicates that the prooxidant was formed during randomization of the purified corn oil, but was not active without trace of metal present. This suggests that the precursor of the formed prooxidant is not a polar compound (such as DAG, MAG, tocopherols) that can be removed by alumina purification from the oil. The prooxidant is likely formed during the randomization reaction of TAG. These results also suggest that the alumina treatment could remove or significantly reduce the iron or copper ions in the oil.

Effect of β-keto ester on oil oxidation. According to a recent study of Liu (2004), β-keto ester could be formed during interesterification. The possible pooxidant effect of β-keto ester was tested using ethyl acetoacetate and ethyl diacetoacetate by OSI. No significant prooxidant effect was found with up to 500 ppm ethyl acetoacetate in natural corn oil. Ethyl diacetoacetate had some prooxidant effect at the level of 500 – 5000 ppm. Acetylacetone was also tested because it is known to complex with metals. At times, there was some prooxidant effect associated with acetylacetone, but the reproducibility was poor. However, when 0.16 ppm cupric ion was added, the OSI time of corn oil treated with all of these three substances did not change significantly. Thus, these compounds may have some prooxidant activity, but do not account for the prooxidant effect we observed in randomized corn oil.

Effect of adsorptive bleaching treatment on the oxidation of randomized corn oil. In industrial refining, interesterified oil may go through a bleaching treatment. To examine if a bleaching treatment would affect the stability of randomized oil, bleached natural or

randomized corn oils were tested by OSI at 100 °C with or without 0.06 ppm copper addition. The results are summarized in Table 13. Bleaching did not make the randomized oil more stable, on the contrary, it made randomized corn oil oxidize a little faster than the non-treated oil, which was probably caused by a tocopherol loss (about 10%) during bleaching treatment (Duff, 1991). When 0.06 ppm cupric ion was added, the randomized oil oxidized much faster, while the OSI time of corn oil did not change appreciably. The OSI time of bleached natural corn oil decreased greatly with cupric ion addition (from 18.55 h to 8.78 h), and the OSI time of randomized and then bleached oil was also greatly decreased with copper addition (from 7.93 h to 1.30 h at 100 °C). These results suggest that bleaching may produce some prooxidants, and it could make the prooxidant problem of randomized oil even worse.

This is the first study to report prooxidant formation during randomization. Previous researchers have used randomization or interesterification as a mean to study the structural effect of TAG on oxidation and obtained inconsistent results, but none of them reported a prooxidant produced by the randomization reaction. This may be caused by several factors, such as the purification method used, the presence of metal ions and the method used to evaluate oil oxidation. The trisaturated and triunsaturated TAGs used by Raghuveer and Hammond (1967) were synthesized from fatty acid methyl esters and triacetin by transesterification. Although the prooxidant should be produced during such a reaction, it might not be active without metal ions present. The dramatically decreased oxidation rate of randomized triunsaturated TAG (0.5-1.5%) in tridecanion could be attributed to better dispersion of unsaturated fatty acid in the TAGs. In fact, they did observe an increase in the oxidation rate of natural vegetable oils (cocoa butter, borneo tallow, soybean and corn oil) after randomization, but they thought this was caused by exposure of unsaturated fatty acids

at the sn-1, 2 positions of glycerol and did not think about the possible presence of a prooxidant. Wada and Koizumi (1983) used 99% pure TAGs in their study and their randomized TAGs were purified by silica acid chromatography. There was probably no metal ions present in their materials and possibly the prooxidant produced by randomization was removed by their purification method. Park et al. (1983a) noticed the increased oxidation rate of randomized soybean oil before purification, but attributed it to reduced tocopherol content in the randomized oil. In the study by Neff et al. (1994) on the oxidative stability of blends and interesterified blends of soybean oil and palm oil, the prooxidant produced by interesterification may have been removed by the purification step using activated carbon and solid phase extraction, since the interesterifed blends did not oxidize faster than soybean oil after purification. Kim et al. (1988) purified the mixture and interesterified mixture of soybean:plam oil (1:1) and soybean:coconut oil (1:1) through a Florisil column. The prooxidant may have been removed since they found no difference in the oxidation of these mixtures before and after interesterification when heating at 180 °C for 50 h. However, this high temperature and the methods used to evaluate oxidation may not have reflected the actual effect of structural change of TAG on its oxidation.

Lau et al. (1982) found that randomized corn oil oxidized 3-4 times faster than natural corn oil after alumina column purification. This is probably because of an incomplete purification in their study. In our study, the oil was eluted from alumina by three column volumes of 5% diethyl ether in hexane to ensure the complete removal of impurities, and HPLC analysis showed no tocopherol, MAG or DAG was detected in the purified oil. The purification procedure was not described in detail in Lau's paper, but she referred to the method of Jensen et al. (1966). In this method, TAG was eluted with 200 mL of 10% diethyl

ether in hexane per 10 g of oil and 20 g of alumina, which was approximately ten column volumes of the solvent. It is very likely that some tocopherols and DAG could be eluted by such large amounts of solvent with twice the polarity that we used. As noted in our study, the prooxidant formed during randomization has a polarity similar to MAG and DAG, the prooxidant could be co-eluted in Lau's purification process and make the randomized oil oxidize faster than natural corn oil. Some evidence for this possibility is that their oxidation rate constant ($days^{-1}$) at 28 °C for natural (0.06 – 0.08) and randomized corn oil (0.18-0.29) after purification was much lower than the oxidation rate (0.80 – 1.29) determined in the current study under the same condition, indicating that some antioxidants must have been present in Lau's purified oils.

GENERAL CONCLUSIONS

The oxidative stability of randomized corn oil with or without purification was investigated at both low (28 – 50 °C) and high (100 °C) temperatures.

Randomized corn oil without purification oxidized much faster than natural corn oil. After purification, there was no difference in the oxidation rate between randomized and natural corn oil. The prooxidant produced during randomization can be removed by an alumina purification process.

The polarity of the prooxidant was similar to MAG and DAG. Although linoleate MAG and DAG showed a slight prooxidant effect in purified corn oil, they were not the prooxidants responsible for the marked increase in the oxidation rate of unpurified randomized oil.

The prooxidant produced during randomization was active only in the presence of trace amounts of metal ions, and the prooxidant activity found in randomized corn oil was greatly increased by the addition of cupric and ferric ions. The oxidative stability of randomized corn oil could be restored by chelating agents, such as EDTA or citric acid. Adsorptive bleaching treatment did not remove the prooxidant from randomized corn oil, but made the oil less stable towards oxidation.

Based on these results, it seems that the prooxidant produced during randomization can facilitate the catalytic activity of metal ions in oil oxidation, but it does not have prooxidant activity by itself. EDTA or citric acid can restore the stability of randomized corn oil, which is probably because of their ability to chelate metal ions and make them unavailable to catalyze oxidation.

According to the enolate ion formation mechanism for interesterification, glyceryl β-keto esters could be formed as intermediates. The glyceryl mono β-keto ester should have a polarity similar to MAG, and if an additional fatty acid is esterified on one of the hydroxyl groups in glycerol, the mono β-keto DAG should have a polarity similar to DAG. The carbonyl and hydroxyl groups in the glyceryl β-keto esters are known to be able to coordinate to transition metals, which can either facilitate their catalytic activity or make them less effective. It is possible that the glyceryl β-keto esters are the prooxidants formed during randomization, if they can complex with metal ions and make them more potent in catalyzing oil oxidation.

Although the results from ethyl acetoacetate and ethyl diacetoacetate did not show the same prooxidant effect as found in randomized corn oil, it should be noted that these two substances are not glyceryl β-keto esters. We are not sure if the hydroxyl groups in glycerol cooperate with the carbonyl groups in complexing with transition metals. It would be necessary to synthesize some glyceryl β-keto esters and test their effects on oil oxidation.

In conclusion, randomization produced some prooxidant that can facilitate the catalytic activity of metal ions in oil oxidation. Structure of the prooxidant is yet to be elucidated. Purification is needed to remove such prooxidant and metal ions. Alternatively, EDTA or citric acid can be added to the randomized oil to prevent the prooxidant activity. Precautions should be taken in the food industry when using interesterificaiton or randomization process to make plastic fats or zero-*trans* margarine.

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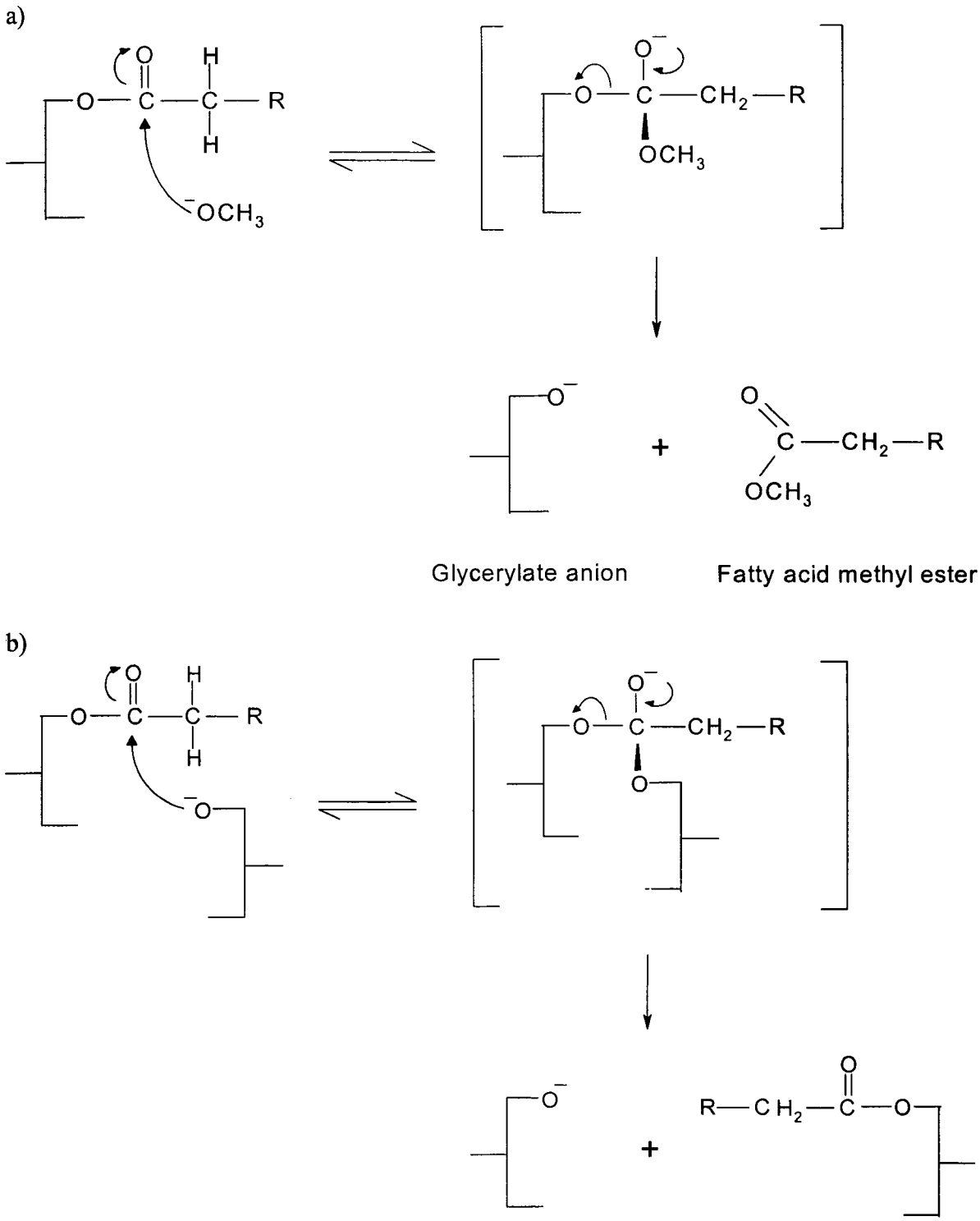

Figure 1. Carbonyl addition mechanism for interesterification

a) Formation of glycerlyate anion; b) Interchange of acyl group.

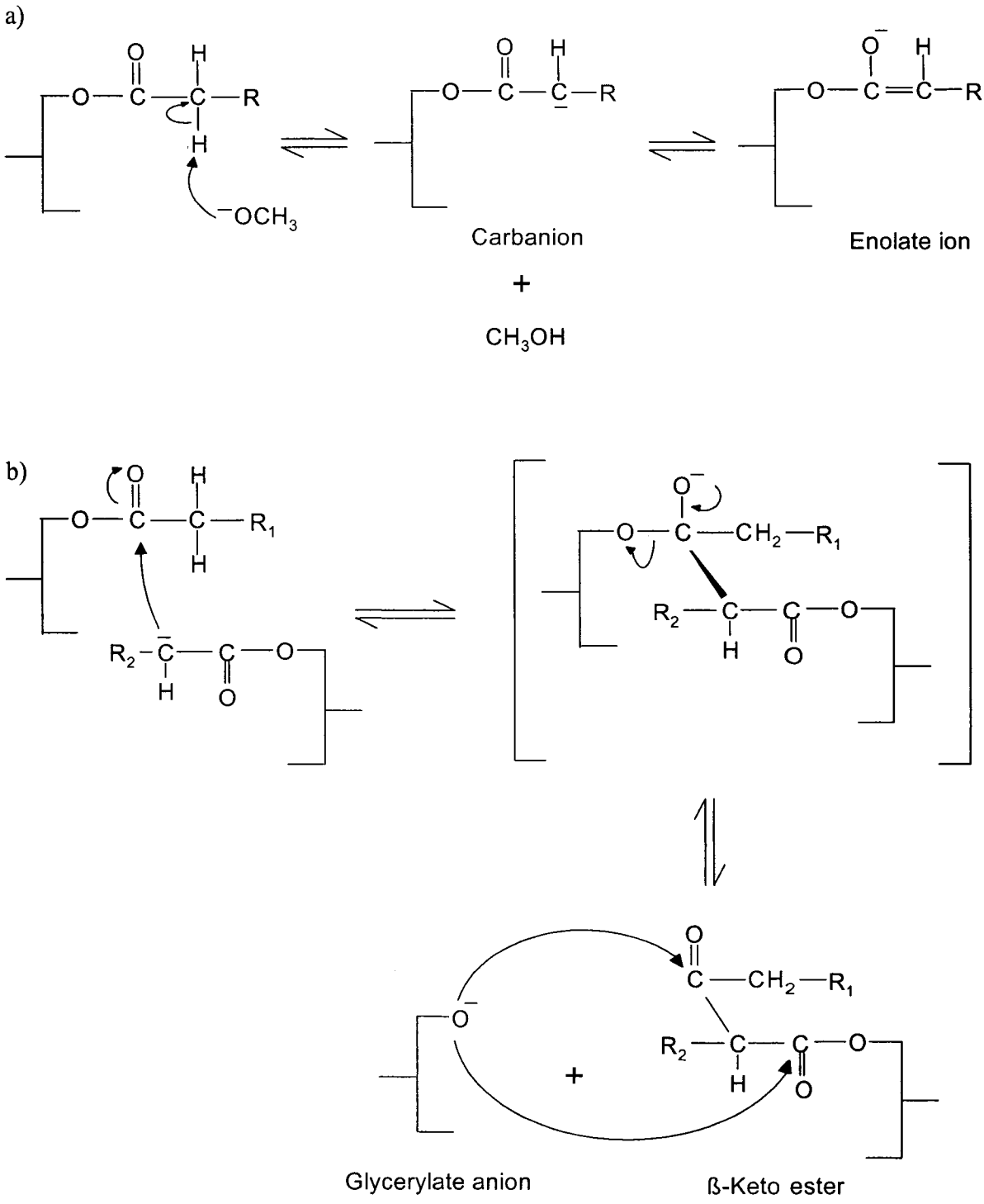

Figure 2. Enolate ion formation mechanism for interesterification

a) Formation of enolate ion; b) Formation of β-keto ester and acyl group interchange.

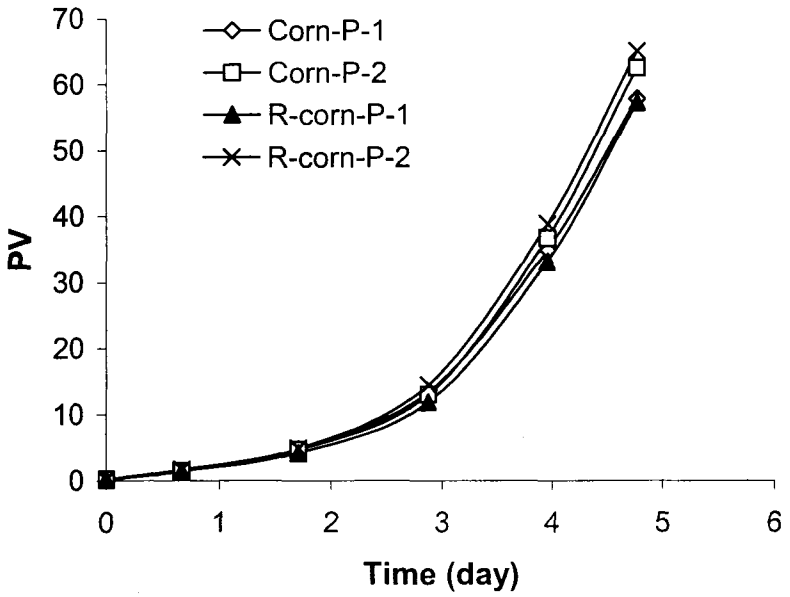

Figure 3. Effect of randomization on the oxidation of purified oil at 28 °C.

P: alumina purified; R: randomized; R-corn-P: randomized and then purified corn oil; 1- and 2- indicate two replicates.

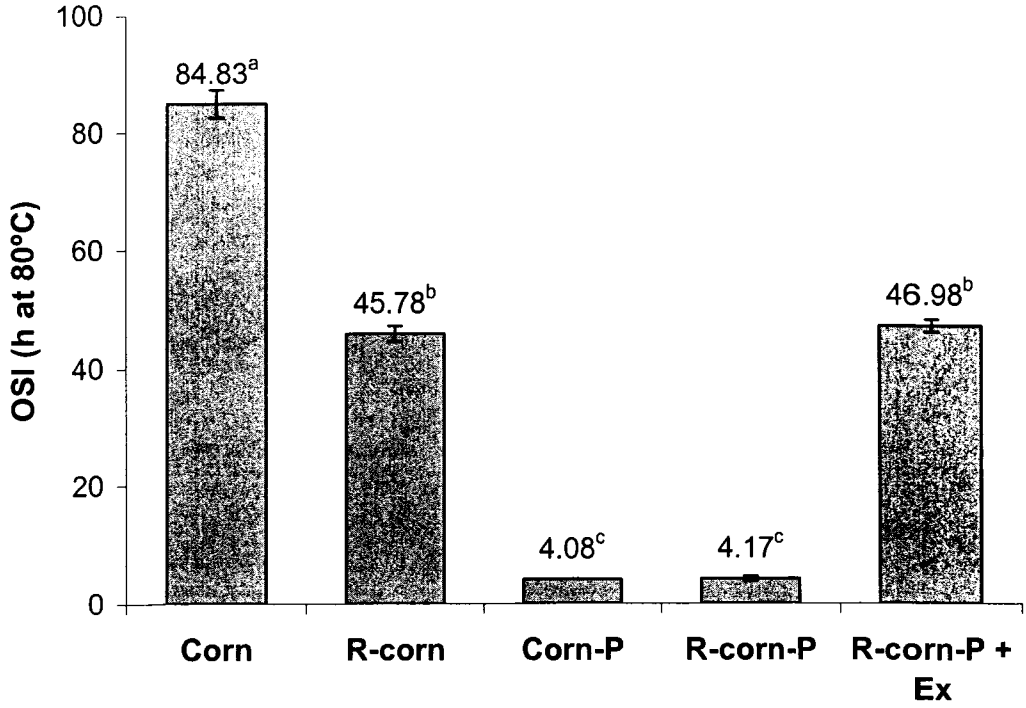

Figure 4. Effect of column purification on corn oil oxidation by OSI at 80°C.

Values not sharing a common superscript are significantly different ($P < 0.05$).

Corn: corn oil; R-corn: randomized corn oil; Corn-P: purified corn oil; R-corn-P: randomized and then purified corn oil; Ex: column extract eluted by methanol from the alumina used to purify randomized corn oil.

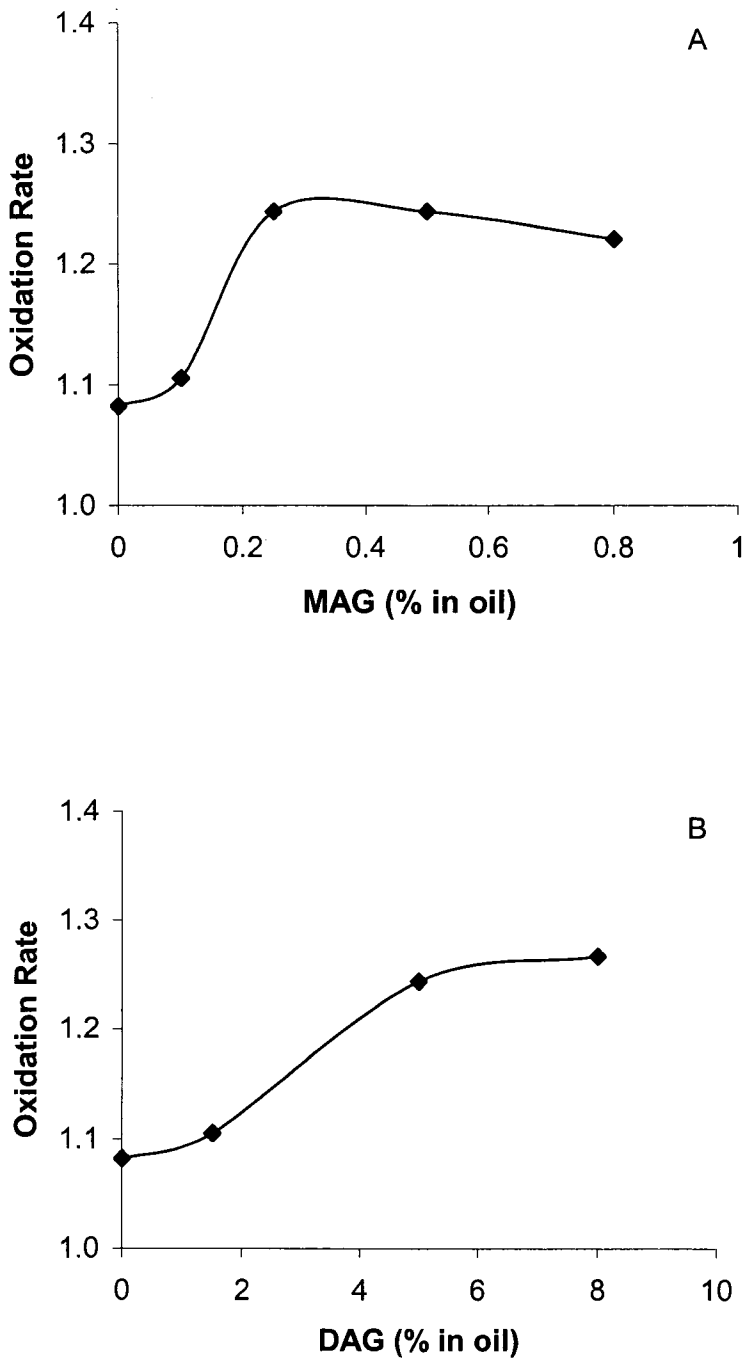

Figure 5. Effect of mono- and di- linoleoyl glycerol (18:2 MAG and DAG) on oxidation of purified corn oil at 28°C.

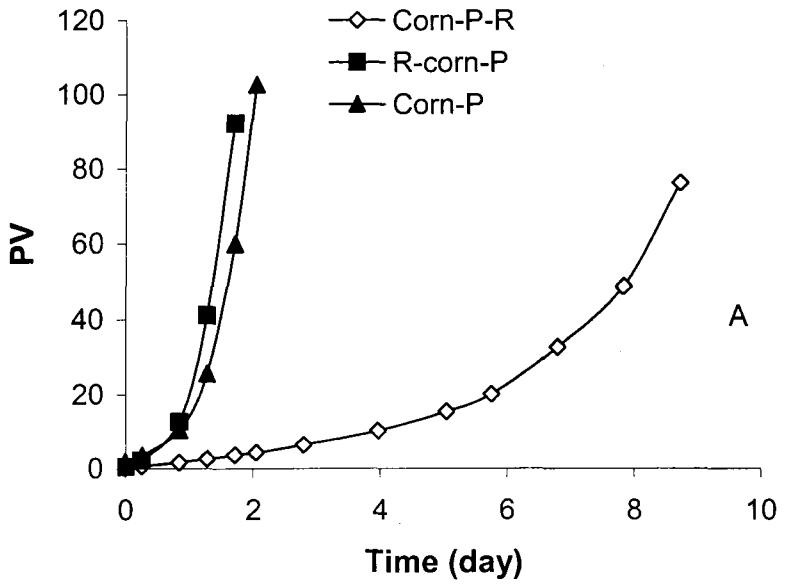

Figure 6. Oxidation of purified and then randomized corn oil (Corn-P-R) at 40°C compared with various controls.

Corn-P: purified corn oil; R-corn-P: randomized and then purified corn oil; Corn-P-R-P: purified Corn-P-R; Corn-P + γ-TP: purified corn oil with 6.6ppm γ-tocopherol.

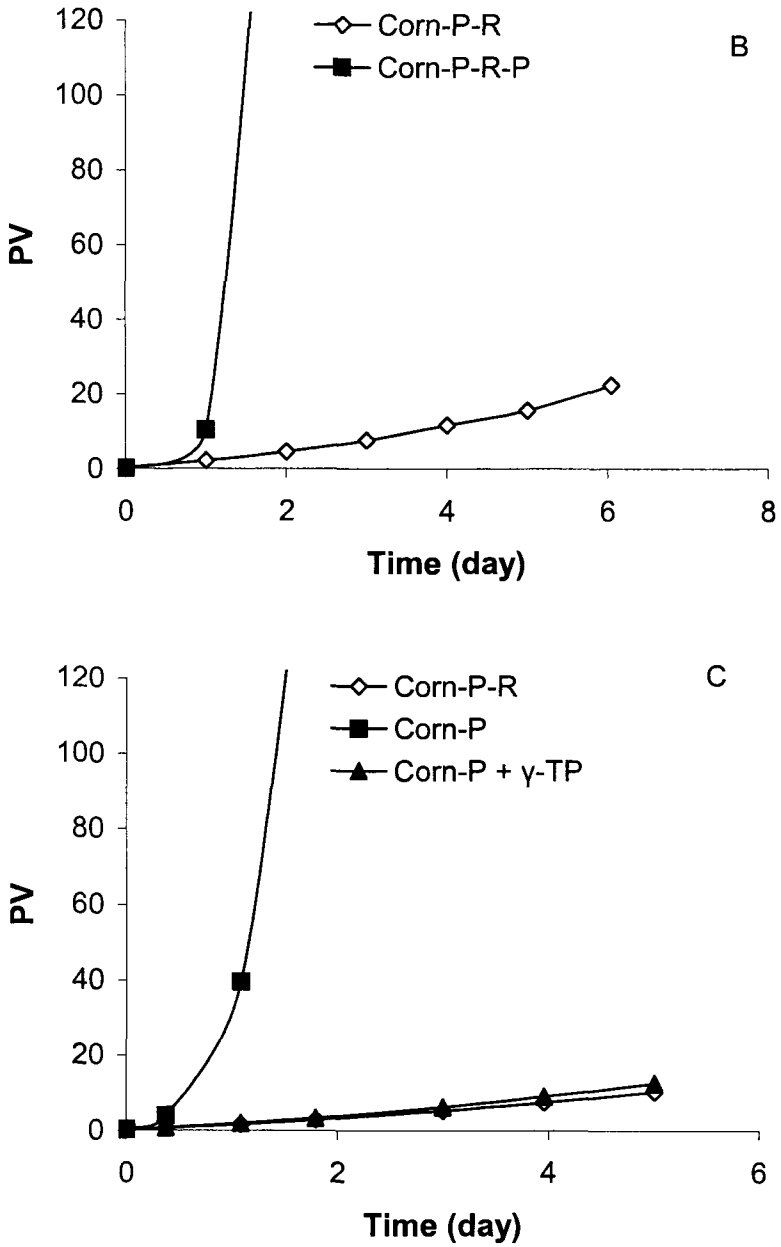

Figure 6. (Continued)

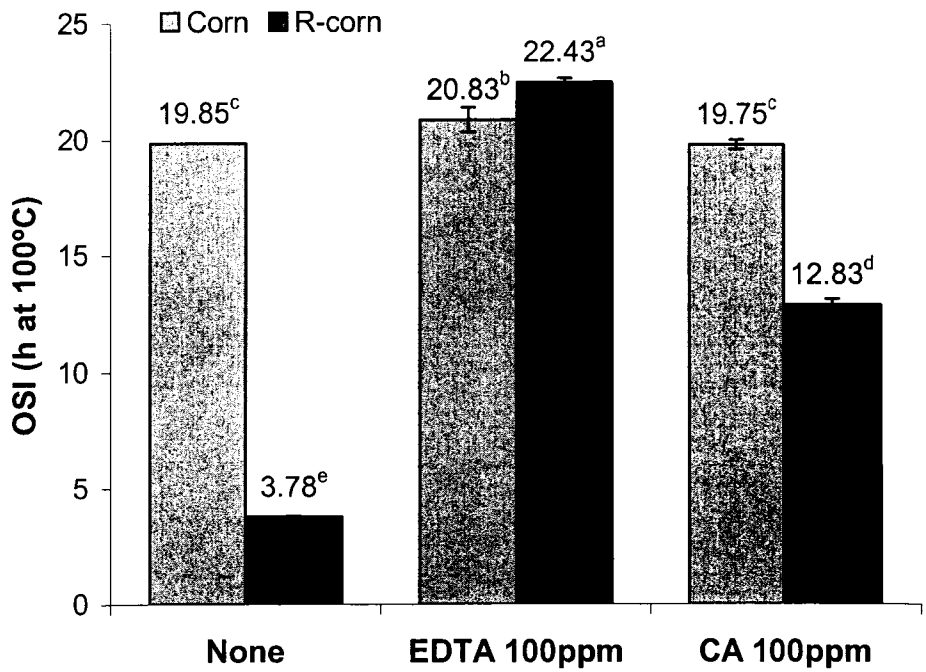

Figure 7. Effect of EDTA and citric acid on the oxidation of natural and randomized corn oil. Values not sharing a common superscript are significantly different ($P < 0.05$). Corn: corn oil; R-corn: randomized corn oil; EDAT: ethylene diamine tetraacitic acid; CA: citric acid

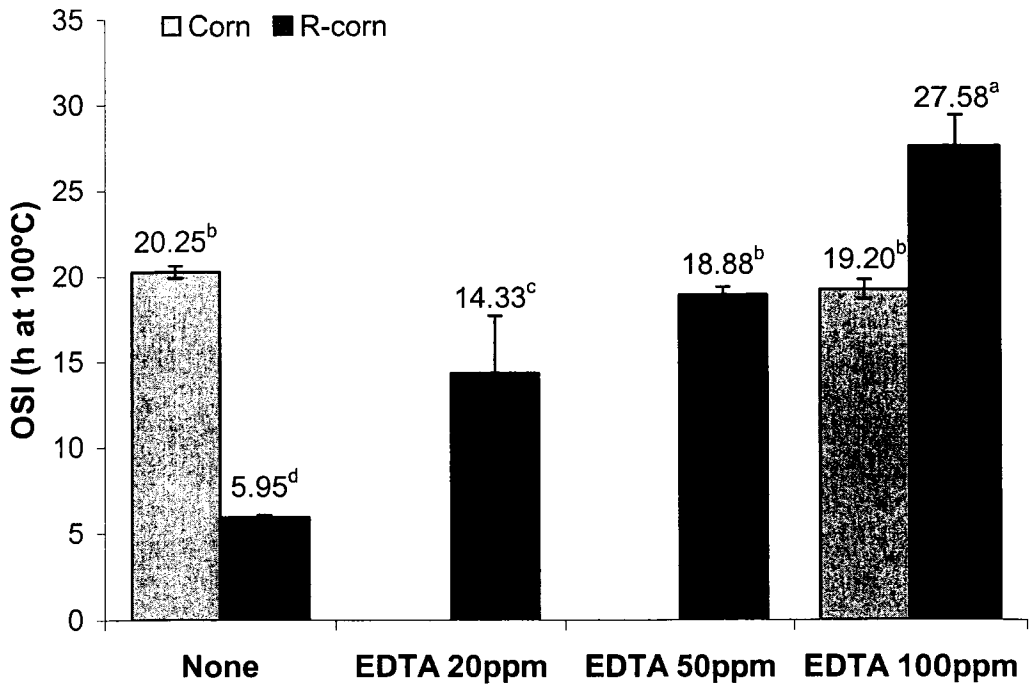

Figure 8. Effect of EDTA on the oxidation of natural and randomized corn oil.

Values not sharing a common superscript are significantly different ($P < 0.05$).

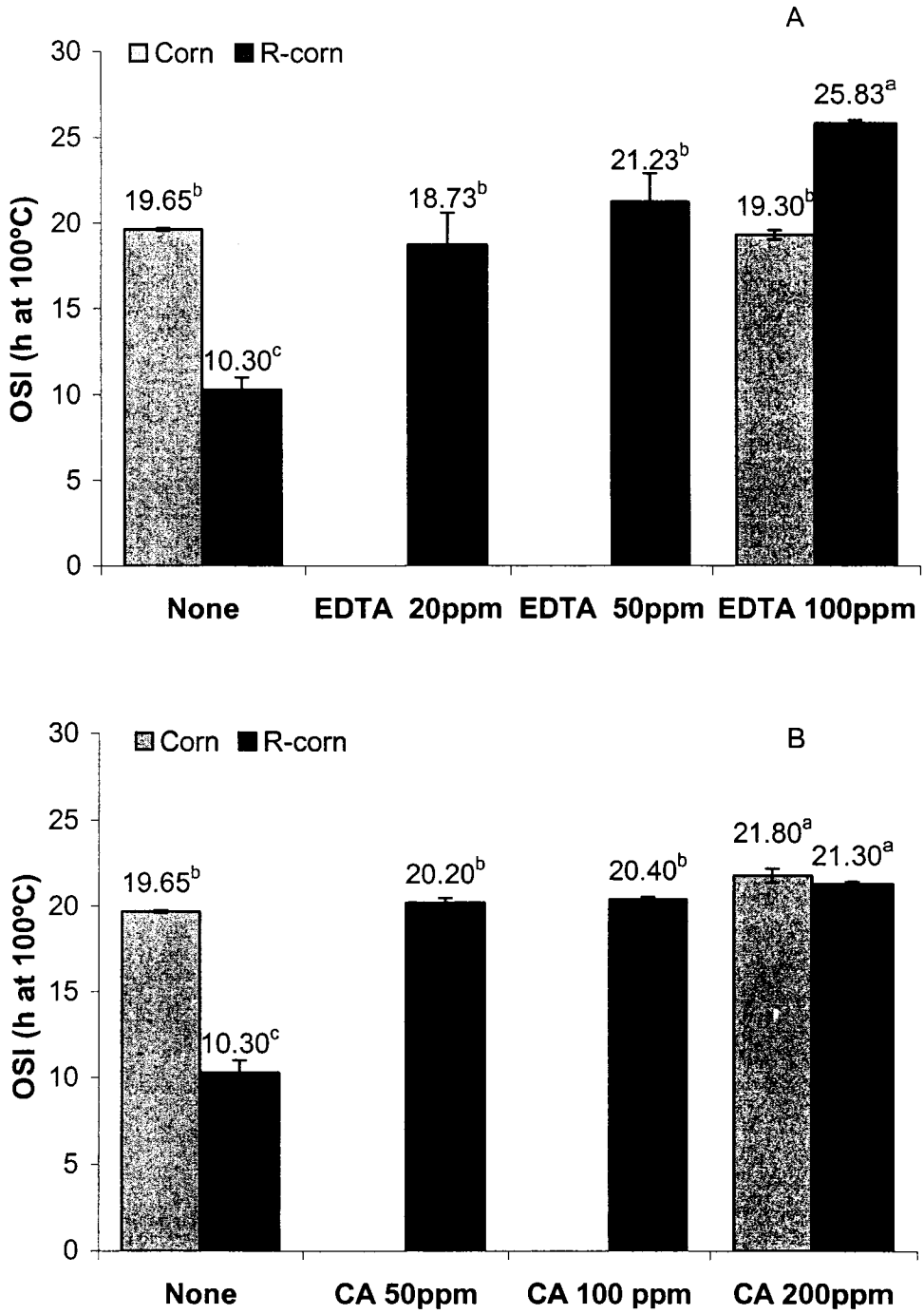

Figure 9. Effect of different levels of EDTA and citric acid (CA) on the oxidation of natural and randomized corn oil.

Values not sharing a common superscript are significantly different ($P < 0.05$).

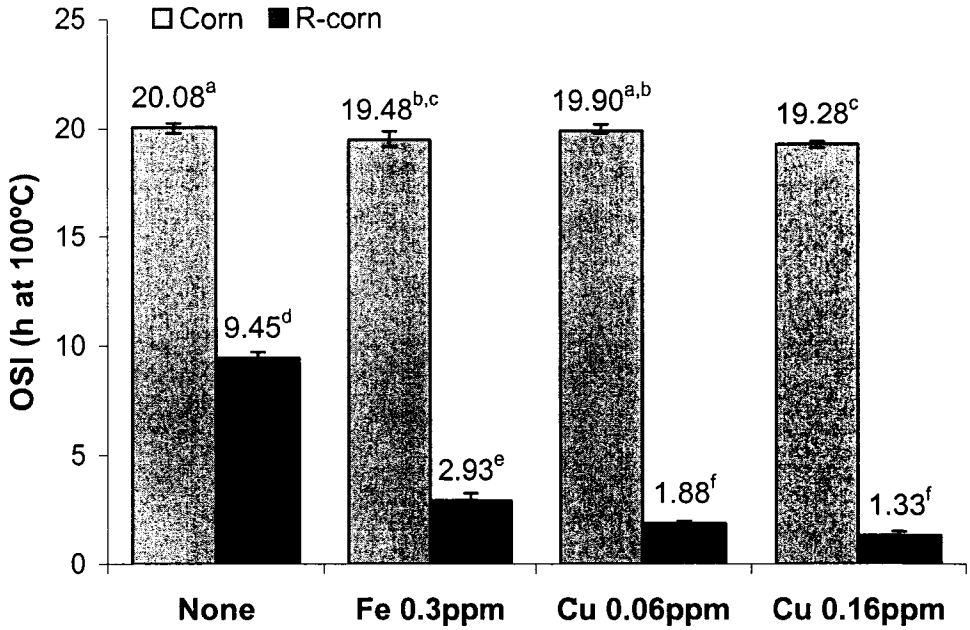

Figure 10. Effect of iron and copper on the oxidation of randomized corn oil by OSI. Values not sharing a common superscript are significantly different ($P < 0.05$). Fe: from Ferric ammonium sulfate; Cu: from cupric acetate

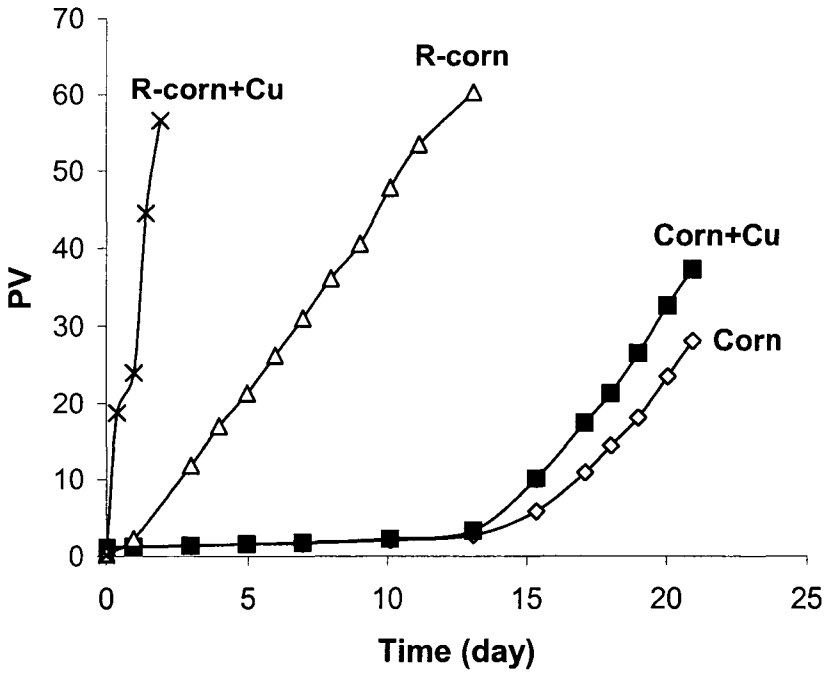

Figure 11. Effect of copper on the oxidation of corn and randomized corn oil at 50 °C. Corn: corn oil; R-corn: randomized corn oil; Cu: 0.15 ppm, from cupric acetate.

Table 1. The mobile phase gradient program for mono- and di-acylglycerol analysis[1, 2]

		% of each channel[2]	
Time (min)	Flow (mL/min)	A	B
0	0.7	98	2
12	0.7	65	35
12.5	0.7	2	98
22	0.7	2	98
22.1	0.7	98	2
29.1	0.7	98	2

[1]Column: 150 x 3.0 mm, 10 μ Chromegasphere SI-60

[2]Channel A, hexane; channel B, hexane/2-propanol/ethyl acetate/10% formic acid (80/10/10/1, v/v/v/v)

Table 2. Fatty acid composition (%) at the *sn*-2 position of natural and randomized corn oil and the overall fatty acid composition of the natural oil

	C16:0	C18:0	C18:1	C18:2	C18:3
Corn oil, overall	10.53	2.07	30.77	55.32	1.30
Corn oil, at *sn*-2	1.66	0.32	28.72	67.71	1.41
Randomized oil, at *sn*-2	11.72	2.17	29.65	55.18	1.28

Table 3. Oxidation rates of various lots of purified natural and randomized corn oils at 28°C[1]

Oil type	Natural	Randomized	Main effect[2]
Lot 1	0.90	0.91	0.91
Lot 2	0.79	0.92	0.83
Lot 3	0.93	0.80	0.86
Lot 4	1.08	1.11	1.10
Lot 5	1.26	1.19	1.22
Lot 6	1.29	1.24	1.27
Main effect[2]	1.01	1.01	

[1]Values are means of two replicates.

[2]The $LSD_{0.05}$ values for comparing different lots of oil and different types of oil (natural vs. randomized) are 0.06 and 0.03, respectively.

Table 4. Oxidative stability by OSI (h at 100°C) of 4 lots of unpurified natural and randomized corn oils[1]

Oil type	Natural	Randomized	Main effect[2]
Lot 1	18.93	10.67	14.80
Lot 2	20.70	3.60	12.15
Lot 3	20.80	5.73	13.27
Lot 4	20.08	9.45	14.77
Main effect[2]	20.13	7.36	

[1]Values are means of two replicates.

[2]The $LSD_{0.05}$ values for comparing different lots of oil and different types of oil (natural vs. randomized) are 0.31 and 0.22, respectively.

Table 5. Effect of sodium ions on the OSI time of corn oil at 100°C [1, 2]

	OSI h at 100°C, mean ± SD	
	Sodium Oleate	Sodium Acetate
Corn	20.64 ± 0.23 [a]	21.30 ± 0.57 [a]
R-corn	5.68 ± 0.07 [d]	10.85 ± 0.14 [e]
Corn + Na 10 ppm	18.81 ± 0.11 [b]	18.04 ± 0.01 [b]
Corn + Na 20 ppm	16.58 ± 0.64 [c]	16.37 ± 1.04 [c]
Corn + Na 50 ppm	16.71 ± 0.88 [c]	12.91 ± 1.17 [d]
Corn + Na 75 ppm	16.87 ± 0.62 [c]	N/A
Corn + Na 100 ppm	16.63 ± 0.92 [c]	15.76 ± 0.18 [c]
Corn + Na 500 ppm	N/A	13.48 ± 0.32 [d]

[1]Values in a column not sharing a common superscript are significantly different ($P < 0.05$); n = 2.

[2]Corn: natural corn oil; R-corn: randomized corn oil.

Table 6. Effect of different levels of tocopherols (Tocs) on oil oxidation by OSI at 100°C [1, 2]

	OSI h at 100°C, mean ± SD
Corn	19.60 ± 0.14 [b]
Corn + 100% Toc	21.80 ± 0.49 [a]
Corn + 60% Toc	21.25 ± 0.14 [a]
Corn-P + 100% Toc	14.60 ± 0.00 [c]
Corn-P + 90% Toc	14.40 ± 0.35 [c]
Corn-P + 60% Toc	13.20 ± 0.00 [d]
Corn-P + 30% Tcoc	10.48 ± 0.04 [e]

[1]Values not sharing a common superscript are significantly different ($P < 0.05$), n = 2.

[2]100% Toc: α- 230 ppm, γ- 670 ppm, δ- 35 ppm as in natural corn oil; for 90% Toc, α- were reduced to 150ppm; 60%, 30% Toc: the same proportions as in the natural corn oil; Corn: corn oil; Corn-P: purified corn oil.

Table 7. Effect of various TLC components isolated from randomized corn oil on oil oxidation by OSI at 100°C [1, 2, 3]

	OSI h at 100°C, mean ± SD
Corn	19.67 ± 1.58 [a]
R-corn	10.88 ± 0.21 [b]
Corn + Ex	12.17 ± 2.60 [b]
Corn + MAG band	10.13 ± 2.69 [b]
Corn + DAG band	13.77 ± 0.62 [b]
Corn + TAG band	19.90 ± 0.07 [a]

[1]Values not sharing a common superscript are significantly different ($P < 0.05$), $n = 2$.

[2]Ex: column extract eluted by methanol form the alumina used to purify randomized corn oil.

[3]MAG, DAG, TAG band: MAG, DAG and TAG separated on TLC, then extracted by methanol.

Table 8. Effect of monolinoleoylglycerol (18:2 MAG) on oil oxidation by OSI at 100°C[1]

	OSI h at 100°C, mean ± SD
Corn	20.33± 0.04 [a]
R-corn	5.88± 0.11 [b]
Corn + 0.1% MAG	20.63± 0.04 [a]
Corn + 0.25% MAG	20.70± 0.49 [a]
Corn + 0.5% MAG	20.60± 0.14 [a]

[1]Values not sharing a common superscript are significantly different ($P < 0.05$), $n = 2$.

Table 9. Effect of saturated MAG, DAG on oil oxidation by OSI at 100°C [1, 2]

	OSI h at 100°C, mean ± SD
Corn	19.25± 0.57 [b, c]
R-corn	10.88± 0.60 [f]
Corn + 0.5% MAG	18.40± 0.35 [c, d]
Corn + 1% MAG	18.10± 0.99 [d]
Corn + 1.5% MAG	14.43± 0.11 [e]
Corn + 2% MAG	14.03± 0.25 [e]
Corn + 0.5% DAG	20.88± 0.53 [a]
Corn + 1% DAG	20.48± 0.11 [a]
Corn + 0.5% MAG + 0.5% DAG	19.88± 0.04 [a, b]

[1]Values not sharing a common superscript are significantly different ($P < 0.05$), $n = 2$.

[2]MAG: 1-Monostearoyl-*rac*-glycerol (Sigma); DAG: Dipalmitin (Nu-Check-PREP).

Table 10. Effect of column extracts on oil oxidation by OSI at 100°C[1]

	OSI h at 100°C, mean ± SD
Corn	20.08 ± 0.25
R-corn	5.90 ± 0.14
Corn + Ex-corn[2]	15.88 ± 0.25
Corn + Ex-R-corn[3]	14.28 ± 0.11

[1]Values are not true replicates, SD is for the OSI measurements.

[2]Ex-corn: column extract eluted by methanol from the alumina used to purify natural corn oil.

[3]Ex-R-corn: column extract eluted by methanol from the alumina used to purify randomized corn oil.

Table 11. Effect of "randomized" triacetin on oil oxidation[1]

	OSI (h at 100°C, mean ± SD)
Corn + Tri	30.37 ± 0.33 [a]
Corn + Tri-Ex1	24.23 ± 0.92 [c]
Corn + Tri-rs1	29.89 ± 0.13 [a, b]
Corn + Tri-Ex2	31.07 ± 0.19 [a]
Corn + Tri-rs2	30.29 ± 0.08 [a, b]
Corn + R-tri	27.75 ± 0.99 [b]
Corn + R-tri-Ex	24.83 ± 2.79 [c]
Corn + R-tri-rs	32.43 ± 0.49 [a]

[1]Values not sharing a common superscript are significantly different ($P < 0.05$), n = 2.

Tri: triacetin; Tri-Ex1: 1st water extract of triacetin

Tri-rs1: residual triacetin after 1st water extraction

Tri-Ex2: water extract of Tri-rs1

Tri-rs2: residual triacetin after the second water extraction

R-tri: "randomized" triacetin using the residual triacetin after 1st water extraction (Tri-rs1)

R-tri-Ex: water extract of randomized triacetin

R-tri-rs: residual randomized triacetin after water extraction

Corn oil and triacetin was mixed in a ratio of 1:1.

Tri-rs1 was added to supplement the weight of water extract of Triacetin.

Table 12. Effect of cupric ion on purified natural and randomized oil oxidation at 28°C [1, 2]

	Oxidation rate (k) Mean ± SD		Oxidation rate (k) Mean ± SD
Corn-P	1.290 ± 0.033 [a]	R-corn-P	1.244 ± 0.065 [a]
Corn-P + Cu	1.370 ± 0.016 [a]	R-corn-P + Cu	1.209 ± 0.016 [a]

[1]Values in a column not sharing a common superscript are significantly different ($P < 0.05$), $n = 2$.

[2]Corn-P: purified corn oil; R-corn-P: randomized and then purified corn oil; Cu: 0.06ppm, from cupric acetate.

Table 13. Effect of bleaching treatment on oil oxidation by OSI at 100°C[1, 2]

	OSI h at 100°C, mean ± SD	
	without Cu	with Cu
Corn	19.10 ± 0.71	18.95 ± 0.07
R-corn	9.78 ± 0.32	2.40 ± 0.14
Corn-B	18.55 ± 0.28	8.78 ± 0.11
R-corn-B	7.93 ± 0.32	1.30 ± 0.00

[1]Values are means of two OSI measurements.

[2]B: bleaching treatment, 2% bleaching earth, 100°C, 20 min in rotary evaporator under vacuum; Corn-B: bleached corn oil; R-corn-B: randomized and then bleached corn oil; Cu: 0.06ppm, from cupric acetate.

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